BS. VET. MED. J. JANUARY 2007 VOL. 17, NO.1, p.61-68

Beni-Suef
Veterinary Medical
Journal

Biochemical changes in lipid peroxidation and anti oxidative defense following lipoic acid administration in alloxan-induced diabetes in rats

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The present study aimed to assess some biochemical changes of oxidative stress in alloxan-induced diabetic rats with administration of lipoic acid. The experiment was carried out on 96 male rats. The group I (32 rats) was left as control (normal non-diabetic). Sixty-four rats were injected subcutaneously with alloxan (120 mg / kg.b.wt.) for induction of diabetes. Then it was divided into two equal groups, group II (diabetic without administration of lipoic acid) and group III (diabetic with administration of lipoic acid). Blood samples were collected from 8 rats of each group for separation of clear serum at 1st, 2nd, 3rd and the 4th week after administration for determination of glucose. Fresh liver and brain tissue samples (0.2 g) were collected from sacrificed rats and homogenized in ten volumes of (ice-cold phosphate saline pH: 7), then kept at –20 ºC for assay of malondialdehyde, reduced glutathione and liver glycogen. The obtained data revealed that serum glucose level was significantly decreased in the 3rd group as compared with the diabetic one. Also there was a significant decrease of liver glycogen in the diabetic group as compared with the non-diabetic control group. Moreover, significant decrease of reduced glutathione in both liver and brain tissues in diabetic group as compared with control. Lipoic acid caused a significant elevation in liver reduced glutathione as compared with the diabetic group, but without effect on brain. There was a significant rise in malondialdehyde in liver and brain tissues of diabetic group as compared with control. On the other hand there was a significant decrease of malondialdehyde in liver and brain tissues of diabetic rats with lipoic acid as compared with diabetic rats throughout the experiment period.

It could be concluded that significant increase of malondialdehyde together with the decrease of reduced glutathione in the diabetic group: indicated the oxidative stress of induced diabetes. Also the study revealed that lipoic acid exerted a powerful antioxidant effect and therefore the diabetics should be supplemented regularly with this vitamin.

Diabetes mellitus is considered as a major health problem allover the world. It has been known in Egypt since the ancient Ebers Papyrus (1550 BC) as a polyuric disease (Kamal, 1967). The free radicals have received a lot of attention in recent years, as they are associated with a variety of pathological events, cancer, aging and diabetes (Baynes, 1991 and Simonian and Coyle, 1996). The free radicals such as superoxide anion (O^-_2), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-) and lipid radical cause DNA damage (Sultana *et al*., 1995) and lipid peroxidation that can lead to cell death (Reiter, 1995). Several hypotheses were suggested to explain the enhanced risks associated with diabetes, among those, is an increase in oxidative stress (Giugliano *et al*., 1995). The oxidative stress may play an important role in the development of complications of diabetes. The most common complications are; retinopathy, nephropathy and neuropathy (Nathan, 1993 and Hong *et al*., 2004). The oxidative stress in diabetes mellitus is mainly due to increased production of free radicals and/or a sharp decline in antioxidant defenses (Low *et al*, 1997). Reactive oxygen species (ROS) can lead to lipid peroxidation and oxidation of some enzymes, as well as protein oxidation

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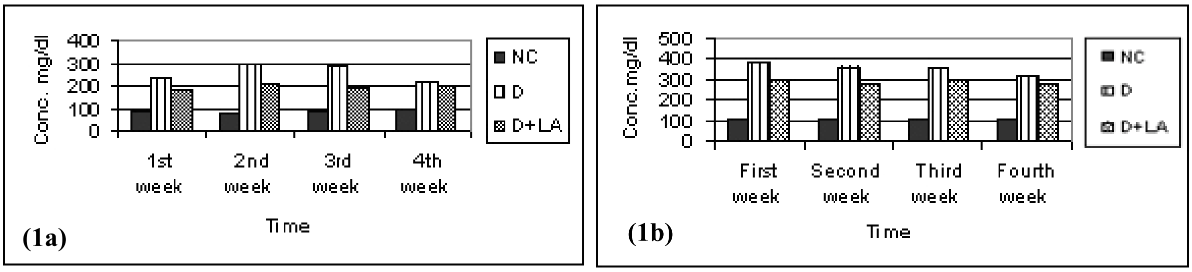


Fig. (1): serum glucose concentration (mg/dl) in normal, diabetic, diabetic with lipoic acid at various periods of time (a) fasting , (b) postprandial.

and degradation. Cells possess several biological systems, defined as scavengers, to protect themselves from the radical-mediated damage (Mates *et al*., 2000). Reduced glutathione (GSH) is a multifunctional tripeptide present in most cells. It directly or indirectly regulates a number of biological processes such as DNA synthesis, ion transport, enzyme activity, transcription, signal transduction and antioxidant defenses (Sen, 2000). Lipoic acid (LA) or thioctic acid is an endogenous sulphur containing free radical scavenger (Cameron and Cotter, 1997). The mechanism of action of LA is increasing de-novo synthesis of cellular glutathione. This occurs by the way of improving cystine utilization through metabolic reduction of LA to dihydrolipoic acid (DHLA) (Han *et al*., 1997). Lipoic acid is found in the mitochondria as a coenzyme of pyruvate and α- ketoglutarate dehydrogenase (Patrick, 2002). LA prevents the increase of lipid peroxide levels but increase GSH levels in some brain tissues in diabetic rats (Baydas *et al*., 2004). So the aim of this work was to clarify the antioxidant effect of lipoic acid and possible scavenge the oxidative stress of diabetic rats.

Materials and Methods

Experimental animals. Ninety six male albino rats were involved in this study with 100-180 g weight and 10 weeks old. Rats were kept for two weeks on balanced ration and water adlibitum for acclimatization.

Alloxan (diabetogenic agent). 5,6-dioxyuracil (Sigma Chemical Company USA) was dissolved in citrate buffer (pH: 4.4) immediately before use.

Lipoic acid (LA). 1,2-dithiolane–3-pentanoic acid, marketed as thioctic acid ® (EVA pharma for pharmaceuticals and medical appliances, Egypt) tablets were crushed and suspended in distilled water.

Experimental diabetes. This was induced in over night fasted rats (16 h.) by a single dose of alloxan (120 mg / Kg.b.wt.) injected S/C. Rats were screened for blood glucose levels (fasting and postprandial) 4-5 days after alloxan injection. Rats that had 180-300 mg/dl serum glucose postprandial level were considered as mid diabetic and included in the experiments (Abdel-Reheim, 1997).

Animal grouping. Three equal groups of 32 rats were used. Group I served as normal non diabetic rats without treatment with lipoic acid. Group II was orally given isotonic solution using stomach tube daily for 4 successive weeks. Group III was treated daily with a dose of 100 mg LA /Kg.bwt by stomach tube for 4 successive weeks.

Sampling and tissue preparations. Blood samples were collected weekly from eight rats of each group (fasting and postprandial). Clear serum samples were obtained for determination of glucose level. After dissection of rats, liver and brain tissues were quickly excised and about 0.2 g of fresh liver and brain tissue were homogenized in ten volumes of ice-cold phosphate saline (pH: 7) until uniform suspension was obtained. The homogenate was kept at –20 °C for determination of thiobarbituric acid reactive substance (TBARS) or malodialdehyde (MDA), GSH and liver glycogen.

Biochemical assay. The serum glucose concentration was estimated enzymatically according to the method of Trinder, (1969). Liver glycogen by the method of Johann and Lentini (1971). MDA concentration in homogenate of liver and brain tissues according to the method described by Albro *et al*. (1986). GSH concentration was determined according to the method of Beutler *et al*. (1963). The obtained data were statistically analyzed according to Snedecor and Cochran (1980).

Results

The obtained data revealed that the serum glucose concentration was significantly decreased in group III as compared with those of

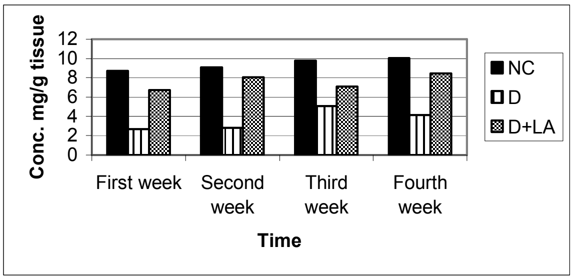


Fig. (2): Liver glycogen concentration (mg/g tissue) in normal, diabetic, diabetic with lipoic acid at various periods of time.

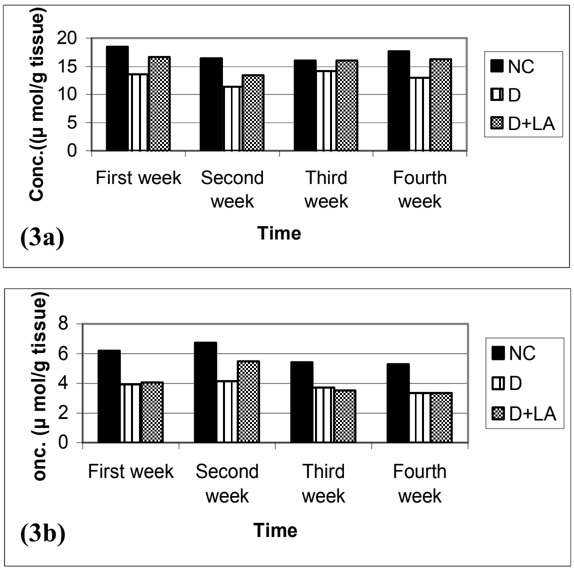


Fig. (3): Reduced glutathione concentration (µ mol/g tissue) in normal, diabetic, diabetic with lipoic acid at various periods of time , (a) liver , (b) brain.

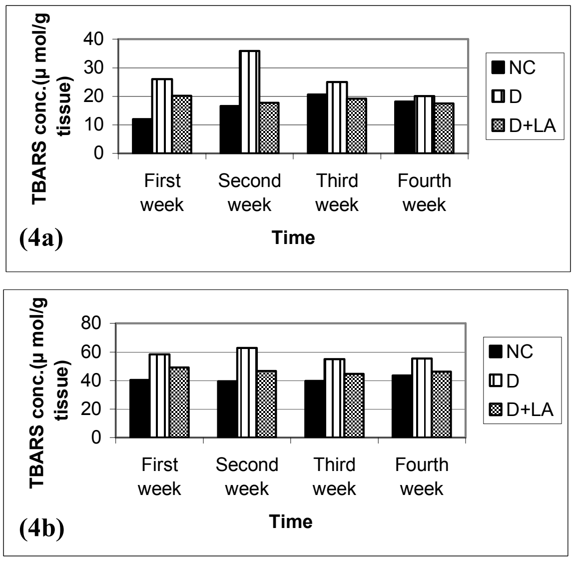


Fig. (4): Thiobarbituric acid reactive substances (TBAS) or malondialdehyde (MDA) concentrate-ion (µ mol/g tissue) in normal, diabetic, diabetic with lipoic acid at various periods of time, (a) liver, (b) brain.

a diabetic one, but significantly higher than the non diabetic throughout the experiment (Table 1, Fig.1 a,b).

The liver glycogen was significantly decreased in diabetic group as compared with normal one all over the period of experiment. On the other hand there was a significant increase in group III as compared with the diabetic one (Table 2, Fig.2).

Both hepatic and brain GSH contents were significantly decreased in diabetic rats as compared with normal one. The results showed a significant increase of both hepatic and brain GSH contents in-group III as compared with diabetic one. Also there was a significant increase in brain GSH at the end of the second week in diabetic rats treated with lipoic acid (Table 3, Fig. 3 a,b).

On the other hand, there was a significant increase in liver and brain MDA at various periods in the diabetic group as compared with normal. In LA-diabetic group there was a significant decrease in both liver and brain MDA contents as compared with diabetic group (Table 4, Fig. 4 a, b).

Discussion

Diabetes mellitus is a frequent metabolic syndrome initially characterized by loss of glucose homeostasis. The disease is progressive and is associated with a high risk of atherosclerosis (Wakabayashi and Masuda, 2004). Alloxan and streptozotocin were widely used as inducers of diabetes mellitus in experimental animals. Both chemicals cause selective destruction of pancreatic islet cells and can induce chronic or permanent diabetes in these animals (Mathe, 1995). Male rats were chosen instead of females in the present study since; males were in general significantly more susceptible to diabetes than females (Paik *et al.*, 1980). The present results showed a significant hyperglycemia in the diabetic group as compared with normal one. This elevation is due to a defect in insulin secretion as in case of insulin dependent diabetes mellitus (IDDM) (Caro, 1990). This also may be attributed to some types of oxygen radicals that attack DNA and thus inducing DNA-strand breaks in β-cells. Such breaks stimulate DNA repair which involve the activation of poly (ADP-ribose) polymerase (PARP), using NAD^+ as a substrate. The fall in NAD^+ can inhibit ATP synthesis, cellular functions as insulin synthesis

Table (1): Serum glucose concentration (mg%) in normal, diabetic and diabetic rat treated with lipoic acid.

Time	Sampling	Group I (NC)	Group II (D)	Group III (D+LA)
First week	Fasting	86.56 ±7.98 [a]	233.42± 13.61 [a]	184.19 ± 14.83 [a]
	Postprandial	104.28 ± 6.62 [b]	381.45 ± 16.47 [b]	302.27 ± 21.50 [b]
Second week	Fasting	83.76 ± 6.45 [a]	292.99 ± 31.15 [a]	209.37 ± 19.81 [a]
	Postprandial	108.92 ± 6.82 [b]	364.82 ± 24.99 [b]	277.48 ± 23.20 [b]
Third week	Fasting	84.92 ± 6.09 [a]	291.03 ± 27.33 [a]	189.37 ± 14.84 [a]
	Postprandial	110.19 ± 9.36 [b]	350.21 ± 28.19 [b]	303.83 ± 24.55 [b]
Fourth week	Fasting	92.04 ± 6.10 [a]	219.33 ± 15.22 [a]	200.68 ± 19.23 [a]
	Postprandial	110.28 ± 8.48 [b]	326.19 ± 25.46 [b]	276.96 ± 26.57 [b]

The LSD at 5% level = 37.07 for fasting sample, and =25.78 for 2.h. pp sample.
The same small letter in the same row means significant difference at ($p < 0.05$).

Table (2): Liver glycogen concentration (mg/gm tissue) in normal, diabetic and diabetic rats treated with lipoic acid.

Time	Group I (NC)	Group II (D)	Group III (D+LA)
First week	8.71 ± 0.61 [a]	2.68 ± 0.12 [a]	6.73 ± 0.42 [a]
Second week	9.10 ± 0.81 [a]	2.81 ± 0.17 [ab]	8.06 ± 0.56 [b]
Third week	9.80 ± 0.61 [a]	5.09 ± 0.41 [a]	7.09 ± 0.58 [a]
Fourth week	10.06 ± 0.70 [a]	4.14 ± 0.30 [a]	8.46 ± 0.61 [a]

The LSD at 5% level = 1.42 .
The same small letter in the same row means significant difference at ($p < 0.05$).

Table (3): Reduced glutathione (GSH) concentration (µmol/gm tissue) in liver and brain tissues of normal, diabetic and diabetic rats treated with lipoic acid.

Time	Sampling	Group I (NC)	Group II (D)	Group III (D+LA)
First week	Liver	18.49 ±0.62 [a]	13.58 ± 0.60 [ab]	16.63 ± 0.89 [b]
	Brain	6.19 ± 0.49 [ab]	3.93 ± 0.25 [a]	4.06 ± 0.27 [b]
Second week	Liver	16.43 ± 0.79 [a]	11.40 ± 1.09 [a]	13.77 ± 0.72 [a]
	Brain	6.74 ± 0.53 [b]	4.15 ± 0.24 [b]	5.47 ± 0.56 [b]
Third week	Liver	16.05 ± 1.47 [a]	14.16 ± 0.66 [c]	16.03 ± 0.71 [b]
	Brain	5.41 ± 0.44 [ab]	3.71 ± 0.42 [b]	3.52 ± 0.19 [a]
Fourth week	Liver	17.68 ± 0.59 [a]	12.97 ± 0.55 [ab]	16.27 ± 0.68 [b]
	Brain	5.29 ± 0.37 [ab]	3.35 ± 0.29 [a]	3.35 ± 0.21 [b]

The LSD at 5% level = 1.93 for liver tissues, and = 1.11 for brain tissues.
The same small letter in the same row means significant difference at ($p < 0.05$).

and secretion, and thus the beta cell ultimately dies (Ohkuwa *et al*., 1995; Pusztai *et al*., 1996). The administration of LA for diabetic rats caused a significant decrease in both fasting and postprandial serum glucose levels as compared with that in the diabetic group. This comes in agreement with the findings obtained by (Khamaisi *et al*., 1999, Kocak *et al*., 2000; Sun and Zhang, 2004). The potent antioxidant effect of lipoic acid as a cofactor for mitochondrial dehydrogenase complexes has been shown to lower blood glucose in diabetic animals presumably through the stimulation of glucose transport by LA (Konrad *et al*., 2001). Moreover, Cho *et al*., (2003) and Bitar *et al*., (2004) postulated that LA activates the insulin-signaling pathway and exerts insulin-like actions in adipose and muscle cells based on the phosphorylation of insulin receptor (IR).

Furthermore, Schroeder *et al*., (2005) attributed the mechanism of action of LA to its blocking effect on interleukin- 1beta that is secreted by activated macrophages in response to immune- mediated process causing islet cell death in IDDM. In addition, Song *et al*., (2005) reported another role of LA in protecting pancreatic β -cell secretory responses by reducing triacylglycerol accumulation in such cells.

In the present study, the liver glycogen content was significantly lowered in diabetic group than in normal one all over the period of study. The decrease in hepatic glycogen of diabetic rats was in agreement with the findings of (Rawi *et al.*, 1998; Abdel-Twab, 2004). This decrease in liver glycogen may be attributed to the enhanced glycogen breakdown, decreased glucokinase, increased glucose - 6- phosphatase activity, increased blood glucose level (Rawi *et al*., 1998) and increased glycogen phosphorylase (Glombitza *et al*., 1994).

Lipoic acid could gradually improve the amount of hepatic glycogen when administered to diabetic rats alone as compared to diabetic rats. The increased glycogen content in liver of diabetic rats after administration of L.A was closely related to the data reported previously by (Jacob *et al*., 1996; Anderwald *et al*., 2002). LA increased both insulin-stimulated glucose oxidation and glycogen synthesis by incorporating glucose into glycogen (Jacob *et al*., 1996).

Both hepatic and brain GSH contents were significantly lower in diabetic rats than in normal non-diabetic ones along the whole period of study. Many reports stated a significant decrease in GSH level in liver and pancreas (Garg *et al*., 1997; Bastar *et al*., 1998) in diabetic rats as compared to normal control.

The depletion of hepatic and brain GSH content in diabetic rats could be attributed to the decreased activity of the key synthesizing enzyme; γ-glutamyle cysteine synthetase. The decrease in the activity of such enzyme could possibly be due to its glycation by uncontrolled hyperglycemia (Jain and McVie, 1994). Another cause of the decreased GSH is the decrease in the activity of glutathione reductase that acts to reduce GS-SG to GSH (Tagami *et al*., 1992; Williamson *et al*., 1993 and Ou *et al*., 1996). All these led to inability of diabetic liver to scavenge the oxidants that may contribute in part to the oxidative stress observed in the diabetic rats. In the present investigation, a remarkable increase in liver GSH levels was shown after treatment of diabetic rats with LA as compared with the diabetic group. The increase in hepatic GSH level after LA administration was found to be in agreement with the previous results reported by (Dincer *et al*., 2002). It was found that LA could increase GSH in many tissues such as kidney (Obrosova *et al*., 2003), lenses (Coppey *et al*., 2001) and brain (Baydas *et al*., 2004). The mechanisms by which LA exerts its antioxidant effect are not completely understood. It may be explained by the thiol nature of lipoate that renders this compound highly reactive against a number of free radicals. Also LA has the ability to regenerate oxidized antioxidants (Packer, 1998).

There was a significant increase in liver and brain MDA at various periods of time in diabetic rats as compared to normal ones. The present data come in agreement with that recorded by (Altomare *et al*. 1992; Ramadan *et al*., 2002; Abdel-Twab, 2004). The significant decrease in both liver and brain MDA concentrations by lipoic acid treatment throughout the tested periods of study as compared to diabetic group was closely related to the finding obtained by (Dincer *et al*., 2002; Baydas *et al*., 2004). This effect can be explained on the basis that LA or its reduced form can prevent lipid peroxidation and protein damage via interaction with vitamin C and glutathione (Packer *et al*., 1995).

It could be finally concluded that, the lipoic acid administration can nearly restore and improve some metabolic changes in carbohydrates metabolism in the alloxan-induced diabetic rats. Another action of LA is its anti-

oxidant effect by donating electrons to the free radicals for neutralizing their reactivities. The combined anti-hyperglycemic and anti-oxidant actions of LA make it useful in preventing many complications of diabetes and become necessary to its uptake as dietary supplement for diabetic persons.

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بعض التغيرات الكيميائية الحيوية فى تأكسد الدهون ومضادات التأكسد المناعية بعد إعطاء حمض الليبويك فى الفئران المصابة بمرض البول السكري التجريبي بواسطة الألو كسان

تهدف هذه الدراسة لتقرير بعض التغيرات الكيميائية الحيوية لحمض الليبويك نتيجة التأثير الأوكسيدى لمرض البول السكري التجريبي بواسطة الألوكسان. أجريت التجربة على عدد ٩٦ فأرا ، قسمت الى ثلاث مجموعات, المجموعة الأولى (٣٢ فأر) تركت كمجموعة طبيعية ضابطة (بدون مرض البول السكري التجريبى) و ٦٤ فأر قد أحدث لهم مرض البول السكري تجريبيا باستخدام مادة الألوكسان بالحقن تحت الجلد (١٢٠ ملجرام/ كجم وزن) ثم بعد ذلك قسمت لمجموعتين : المجموعة الثانية (٣٢ فأر) (مصابة بمرض البول السكري فقط) والمجموعة الثالثة (٣٢ فأر) (مصابة بمرض البول السكري مع إعطائها حمض الليبويك) وقد استمرت التجربة لمدة أربعة أسابيع. تم تجميع عينات الدم من عدد ٨ فئران من كل مجموعة فى نهاية الأسابيع الأول والثاني والثالث والرابع لتقدير مستوى سكر الجلوكوز , وكذلك عينات من الكبد والمخ بعد قتل الفئران. وتم طحن عينات الكبد والمخ مع ١٠ أحجام من محلول ملح فسيولوجي وتحويلها إلى مخلوط متجانس باستخدام الخلاط, حفظت في درجة حرارة – ٢٠م لحين قياس مستويات كل من المالون داى ألدهيد, الجلوتاثيون المختزل في أنسجة الكبد والمخ وكذلك الجليكوجين فى الكبد فقط. وأوضحت النتائج بعد التحليل الإحصائي أن هناك نقص معنوي في مستوى سكر الجلوكوز فى مصل فئران المجموعة الثالثة مقارنة بالمجموعة الثانية. وكذلك كان هناك نقص معنوي فى مستوى الجليكوجين فى أنسجة الكبد في المجموعة الثانية مقارنة بالمجموعة الطبيعية الضابطة. إضافة إلى وجود نقص معنوي في مستوى الجلوتاثيون المختزل في أنسجة الكبد و المخ فى المجموعة الثانية (مصابة بمرض البول السكري فقط) مقارنة بالمجموعة الأولى الضابطة. علاوة على ذلك فإن تأثير حمض الليبويك في المجموعة الثالثة قد تسبب فى زيادة معنوية فى مستوى الجلوتاثيون المختزل في أنسجة الكبد مقارنة بالمجموعة الثانية, بينما لم يكن له تأثير واضح فى الأنسجة المخية. وكذلك كان هناك زيادة معنوية فى مستوى المالون داى ألدهيد في أنسجة الكبد و المخ في الفئران المصابة بمرض البول السكري مع إعطائها حمض الليبويك مقارنة بالمجموعة المصابة بمرض البول السكري فقط خلال فترة إجراء التجربة. ونخلص في النهاية إلى أن الزيادة المعنوية فى مستوى المالون داى ألدهيد والنقص المعنوي في مستوى الجلوتاثيون المختزل في أنسجة الكبد و المخ توضح التأثير الأوكسيدى لمرض البول السكري التجريبي بواسطة الألوكسان. وتبين الدراسة أن حمض الليبويك له تأثير قوى وفعال كمانع أكسدة , كما ينصح بتعاطيه لمرضى البول السكري كعلاج تكميلي.